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Synthesis of Iodinated Biochemical Tools Related to the 2-Azetidinone Class of Cholesterol Absorption Inhibitors

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Abstract—The discoveries of Sch 48461 and Sch 58235 and their novel pharmacology of inhibition of cholesterol absorption have prompted efforts to determine their biological mechanism of action (MOA). To this end, a series of radioiodinated analogues with good to excellent in vivo activity have been designed and synthesized as single enantiomers. They are structurally consistent with the allowable SAR of the 2-azetidinone class of cholesterol absorption inhibitors. © 2002 Elsevier Science Ltd. All rights reserved.

Recently, we described the discovery of a very potent class of cholesterol absorption inhibitors (CAI) typified by the original lead compound in this series, Sch 48461 in Figure 1.[1] Our SAR development in this program revealed the importance of side-chain metabolic hydroxylation to activity and led to the discovery of our current clinical candidate, **2** (Sch 58235, ezetimibe).[2,3] While these 2-azetidinones are extremely potent inhibitors of cholesterol absorption in vivo, the precise biological mechanism by which this inhibition takes place has yet to be discovered.[4] Data presented previously suggest that there is a molecular target for these compounds, which we have designated the Cholesterol Absorption Inhibitor Binding Protein (CAIBP). Relying on the large SAR database that we subsequently developed in this series,[5] we have carefully designed and prepared a number of biochemical tools for the investigation of this mechanism.[6] The scope of these tools include high affinity radioactive or fluorescent analogues for binding/affinity applications, crosslinking reagents for photoaffinity labeling experiments, and biotinylated derivatives for affinity chromatography applications. Our initial approach was to use radiolabeled binding compounds for the identification of subcellular localization sites in intestinal cells.[7] Once we had some knowledge of where the compounds were binding, we hoped to identify any specific proteins that had high affinity for this class of CAIs using other techniques. This paper will discuss the design and preparation of high affinity radiolabeled compounds, while the synthesis of related tools will be presented elsewhere.[8]

Based on our extensive SAR development of the azetidinone CAI series, we knew that the two best structural sites for elaboration while maintaining in vivo activity were the *N*-aryl ring and the pendant aryl ring. For sensitivity reasons, our initial binding studies required radioiodinated analogues and thus the targets **3** and **4** came to the fore. Compound **3** became our first target because its preparation could serve to further our SAR development in that region, proceeding through an intermediate diazonium ion. The compound was prepared in a straightforward manner as shown in Scheme 1.

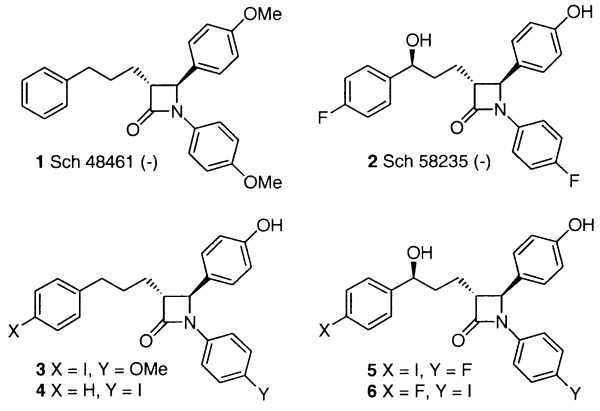

Figure 1. CAI derivatives.

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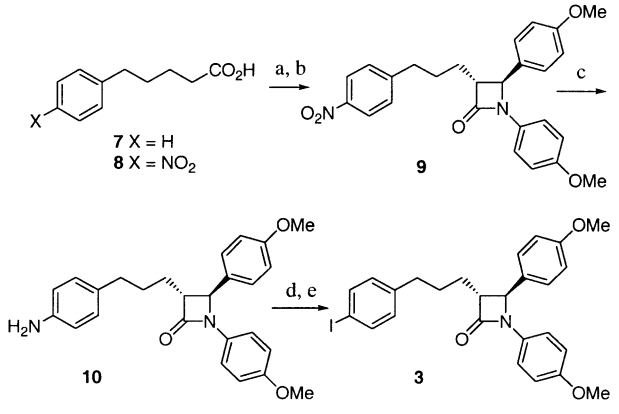
Phenyl valeric acid was nitrated predominately in the *para* position. The resulting acid was converted to its acid chloride and subjected to ketene-imine condensation conditions to generate the requisite *trans* β-lactam.[9] Reduction of the nitro group and subsequent diazotization gave the intermediate diazonium salt, which was not isolated, but converted directly to aryl iodide **3**.[10,11] The regioisomeric aryl iodide **4** was prepared in a somewhat simpler manner using analogous ketene-imine chemistry and the corresponding *p*-methoxybenzylidene-4-iodoaniline (50% yield, 12:1 *trans*/*cis*).

Lacking an in vitro assay to gauge our success at designing molecules with high affinity, our efforts were forced to rely on an in vivo assay of cholesterol absorption to qualitatively assess the pharmacological summation of affinity, absorption, distribution, metabolism, and excretion.[12] We felt confident that if an analogue displayed good in vivo activity, then its affinity for the receptor must be high. The in vivo data for these compounds in our cholesterol fed hamster assay is shown in Table 1. Compounds **3** and **4** were both active in vivo, with the *N*-iodophenyl analogue **4** having slightly better potency.

The assumptions made in our assessment of the affinity of these compounds for the putative CAIBP required that any metabolites formed in vivo be less potent than the parent. In fact, this assumption proved to be false. In our studies leading to the discovery of Sch 58235, we determined that metabolism of Sch 48461 primarily occurred at the C-4 methoxyphenyl ether.[3,13] Demethylation at this site generated the corresponding phenol, which was about equipotent with the parent compound. In addition, oxidation at the benzylic 3′ position to generate the analogous hydroxyl led to derivatives that were as much as two orders of magnitude more potent than the parent. Although this extensive metabolism occurred to only a small percentage in our animal models, the enhanced activity of the metabolites could potentially account for nearly all our in vivo activity. Thus the design of our affinity ligands needed to reflect this discovery. Our next generation of iodinated ligands was therefore designed to resemble the more potent Sch 58235, while introducing the iodine atom at the two previous sites of substitution.



Scheme 1. Preparation of pendant aryliodide analogue, **3**: (a) HNO_3, H_2SO_4, −10 °C (28%); (b) (i) $(COCl)_2$ (ii) ArCH=NAr, Bu_3N, tol, Δ (51%); (c) H_2, Pd/C (97%); (d) (i) HOAc, $NaNO_2$, H_2O; (ii) KI (72%).

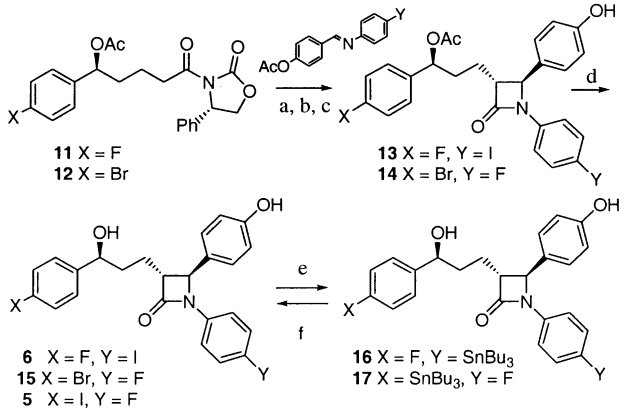
Our synthesis of these iodinated analogues is based on an asymmetric synthesis of Sch 58235 and is shown in Scheme 2.[14] The enantiomerically pure 5′-(*S*)-acetoxy-5′-(4-fluorophenyl)valeroyl-5-(*S*)-phenyloxazolidinone, **11**, was treated with titanium tetrachloride, Hünig's base, and the acetoxy protected imine to give the intermediate β-amino acyloxazolidinone. The major diastereomer was purified to homogeneity by crystallization and then cyclized in two steps by first silylation with bis-trimethylsilylacetamide followed by treatment with a catalytic amount of tetrabutylammonium fluoride. In the workup the phenolic acetate group was lost resulting in a single enantiomerically pure 2-azetidinone, **13**, after crystallization. Base hydrolysis revealed the benzylic hydroxyl group to give the desired product **6**. Analogously, the pendant bromophenylpropyl analogue, **15**, was prepared from the bromophenyl oxazolidinone **12**. In order to generate the radioiodinated versions of these compounds for binding studies, we next converted these two analogues to their tributylstannyl derivatives by palladium mediated stannylation in refluxing toluene. Iodination was achieved by treatment with NaI and Iodobeads® or Iodogen® as a mild oxidant in a pH 5.8 phosphate buffered ethanolic solution. Compounds **5** and **6** could both be prepared efficiently by this method.

Finally, we needed to address the issue of the bio-relevant form of our tools. We had discovered that Sch 58235 exists predominately as its glucuronide in vivo.[3] While this metabolite was not unexpected, in the

Table 1. In vivo activity of CAIs in a cholesterol-fed hamster assay

Compd	% Redn HCE @ dose (mg/kg/day)	ED_{50} (mg/kg/day)
1 (−)	−93@10	2.2
2 (−)	−100@1	0.04
3 (±)	−56@50	na
4 (±)	−82@50	na
5	−54@1	na
6	−63@1	0.49
22	−54@1	na

na, not applicable.

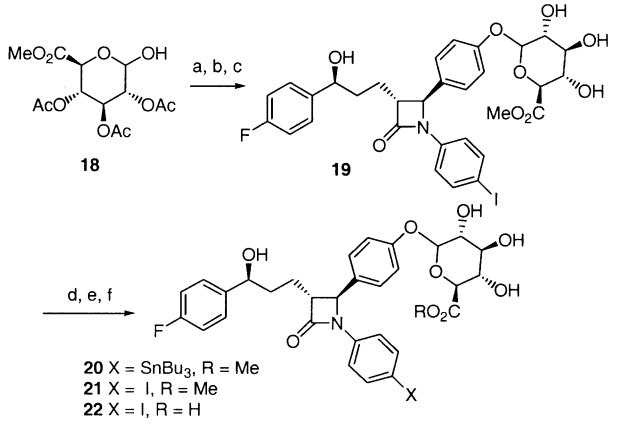


Scheme 2. Synthesis of iodinated analogues of Sch 58235: (a) imine, $TiCl_4$, *i*-Pr_2NEt, CH_2Cl_2 (51%); (b) BSA, toluene; (c) TBAF (cat) (93%); (d) LiOH, THF, H_2O (56%); (e) $(Bu_3Sn)_2$, $Pd(Ph_3P)_4$, toluene, Δ (47%); (f) NaI, iodobeads®, EtOH, pH 5.8 buffer (81%).

absence of a clear understanding of the mechanism of action, we could preclude neither the free phenol nor the glucuronide as the bioactive species. We therefore required the corresponding iodinated glucuronide of our derivative **6** for evaluation in our studies. The obvious choice to minimize the number of 'hot' steps in our synthesis would be to prepare the corresponding glucuronide of the *N*-arylstannane. Efforts along these lines revealed a sensitivity of the arylstannane to the acid moiety of the glucuronide. Thus we prepared the analogous methyl ester **19** by glycosylation of the phenol **13** using BF_3-mediated trichloroimidate chemistry on the fully protected sugar, **18**, as shown in Scheme 3.[13c] Hydrolysis of all the acetate groups with KCN and stannylation of the resulting methyl ester gave the desired arylstannane **20**. Iodination and subsequent hydrolysis of the methyl ester proceeded to **22** without problems. This longer route to the product was sufficient to provide radioiodinated material in high yield and purity, even though it requires the inclusion of a second deprotection step in a 'hot' process.

As can be seen from the data in Table 1, the derivatives bearing the benzylic hydroxyl moiety have a significant potency advantage over compounds **3** and **4**. Consistent with our previous results, the *N*-iodophenyl analogue **6** was slightly more potent than the pendent iodophenyl derivative **5**. Finally, the glucuronide showed significant reduction of hepatic cholesterol esters at 1 mg/kg/day in our cholesterol-fed hamster assay. Compounds **6** and **22** were chosen for radioiodination according to the procedures described above using carrier free sodium iodide resulting in biochemical tools with specific activity of ~200 Ci/mmol.[15] These compounds were suitable for use in all our binding studies and MOA experiments.

We have described the evolution of the design of iodinated analogues of Sch 48461 and Sch 58235 and the synthesis of those analogues. Biological activity was confirmed by in vivo testing of each analogue in order to be assured of significant affinity for the target CAIBP. The most active analogues, **6** and **22**, were prepared as 'hot' biochemical tools for use in binding and localization studies by our biology group. Results from those studies will be presented in due course.



Scheme 3. Preparation of iodinated glucuronide derivative **22**: (a) Cs_2CO_3, Cl_3CCN, CH_2Cl_2 (55%); (b) **13**, BF_3–Et_2O, CH_2Cl_2 (77%); (c) KCN, MeOH (49%); (d) $Bu_3SnSnBu_3$, $Pd(Ph_3P)_4$, toluene, Δ (25%); (e) NaI, Iodogen™, 10:1 EtOAc/HOAc (74%); (f) MeOH, Et_3N, H_2O 1:2:7 (quant).

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10. The intermediate diazonium could also be trapped with other nucleophiles to generate the substituted aryls. This chemistry was exploited as part of our SAR studies. See ref 5.
11. All compounds were characterized by ^1H NMR, MS and/or HRMS where appropriate.

Analytical data: Compound **3**: yellow oil, ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, 2H, $J=8$ Hz), 7.25 (d, 2H, $J=9$ Hz), 7.21 (d, 2H, $J=9$ Hz), 6.91 (d, 2H, $J=9$ Hz), 6.89 (d, 2H, $J=8$ Hz), 6.77 (d, 2H, $J=9$ Hz), 4.54 (d, 1H, $J=2$ Hz, CHN), 3.80 (s, 3H, OMe), 3.74 (s, 3H, OMe), 3.05 (m, 1H, CHCO), 2.59 (t, 2H, $J=7$ Hz), 1.84–1.78 (m, 4H). MS (EI) m/z 527.1 (M^+), Anal. calcd for $C_{26}H_{26}INO_3$: C, 59.21, H, 4.97, N, 2.66%, found: C, 59.44, H, 4.86, N, 2.81%.

Compound **4**: white crystalline solid, mp 96.5–97.5 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, 2H, $J=8.6$ Hz), 7.31–7.14 (m, 7H), 7.03 (d, 2H, $J=8.7$ Hz), 6.89 (d, 2H, $J=8.7$ Hz), 4.56 (d, 1H, $J=2.4$ Hz, CHN), 3.80 (s, 3H, OMe), 3.08 (m, 1H, CHCO), 2.64 (t, 2H, $J=7$ Hz), 1.97–1.79 (m, 4H). MS (CI) m/z 498 $(M+H)^+$, Anal. calcd for $C_{25}H_{24}INO_2$: C, 60.35, H, 4.87, N, 2.82%, found: C, 60.41, H, 4.91, N, 3.09%.

Compound **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, 2H, $J=8$ Hz), 7.25–7.16 (m, 4H), 7.08 (d, 2H, $J=9$ Hz), 6.92 (dd, 2H, $J=8$, 9 Hz), 6.83 (d, 2H, $J=8$ Hz), 5.30 (s, 1H, OH), 4.68 (m, 1H, C**H**OH), 4.55 (d, 1H, $J=2$ Hz, CHN), 3.08 (m, 1H, CHCO), 2.40 (br s, 1H OH), 2.00–1.85 (m, 4H). MS (FAB) m/z 518 $(M+H)^+$.

Compound **6**: white solid, mp 169.5–170.0 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, 2H, $J=8.5$ Hz), 7.28 (dd, 2H, $J=5.5$, 8.8 Hz), 7.18 (d, 2H, $J=8.5$ Hz), 7.04–7.00 (m, 2H), 6.83 (d, 2H, $J=8.5$ Hz), 4.71 (t, 1H, $J=6$ Hz, C**H**OH), 4.56 (d, 1H, $J=2$ Hz, CHN), 3.06 (dt, 1H, $J_d=2$ Hz, $J_t=7$ Hz, CHCO), 2.00–1.85 (m, 4H). MS (FAB) m/z 518 $(M+H)^+$, exact mass calcd for $C_{24}H_{22}FINO_3$ m/z 518.0628, obsd m/z 518.0619.

Compound **22**: beige solid, mp 135.0–137.0 °C, ^{1}H NMR (300 MHz, CD_3OD) δ 7.45 (d, 2H, $J=8$ Hz), 7.22–6.86 (m, 10H), 4.87 (d, 1H, $J=3$ Hz, CHO_2), 4.68 (br s, 1H, CHN), 4.48 (m, 1H, C**H**OH), 3.87 (d, 1H, $J=9$ Hz, $CHCO_2$), 3.53–3.43 (m, 1H), 3.39-3.34 (m, 1H), 3.10 (dd, 1H, $J=7$, 8 Hz), 3.01–2.93 (m, 1H, CHCO), 2.05 (br s, 1H), 1.80–1.69 (m, 1H). MS (FAB) m/z 751 $(M+NaCl)^+$, 717 $(M+H+Na)^+$, 694 $(M+H)^+$.

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